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13. ABSTRACT (Maximum 200 Words)
The primary aim of this clinical study is to determine the relationship between pre-treatment prostate cancer oxygen levels and long-term disease control following treatment with radiotherapy, and the independent prognostic effect of oxygen measurements relative to established prognostic factors. In addition, the study will determine the relationship between pre-treatment tumor oxygen levels and mutations of the p53 tumor suppressor gene, and the impact of this interaction on patient outcome. The accrual rate increased in year 2 and is now close to the value anticipated when the study was designed. Nevertheless, because of slower than expected accrual I year 1 and disruption in clinical and research activity in year 2 by Severe Acute Respiratory Syndrome (SARS), it is anticipated that accrual will need to be extended into year 4 to meet the target of the 195 patients. The oxygen measurement technique was revised based on the results of our pilot study to assure that the highest quality data are being collected. The microregional distribution of oxygen in prostate cancer biopsies will be studied using intrinsic markers of oxygenation. The molecular studies of p53 are proceeding as outlined in the proposal. It is anticipated that all aspects of the proposed work will be completed.

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Introduction

Hypoxia is known to impair the effectiveness of both surgery and radiotherapy at curing a variety of solid human tumors. This may result from hypoxia-induced increase in genetic instability leading to altered expression of genes that are important in tumor growth and progression. These genetic changes are manifested clinically as more aggressive tumor behavior. The primary aim of this clinical study is to determine the relationship between pre-treatment prostate cancer oxygen levels and long-term disease control following treatment with radiotherapy, and the independent prognostic effect of oxygen measurements relative to established prognostic factors. In addition, the study will determine the relationship between pre-treatment tumor oxygen levels and mutations of the p53 tumor suppressor gene, and the impact of this interaction on patient outcome.

Body

Progress in the second year of the research award has focused on Task 1 of the Statement of Work:

Task 1. Accrual of patients (years 1-3)

Patients will be accrued to this study at a uniform rate of 65 per year (52 eligible patients per year allowing for 20% attrition), over the three years from January 2001 to December 2003. Clinical and surgico-pathologic prognostic information will be collected prospectively at the time each patient enters the study. Eppendorf oxygen measurements will be made. A biopsy will be obtained immediately after the oxygen measurements and evaluated for mutations of the p53 tumor suppressor gene and apoptosis. The biopsies will be processed in batches during the accrual period. A portion of each biopsy will be stored for future study of other hypoxia-related genes.

Patients will be accrued to the EF-5 component of the study in the second and third years once phase I testing of this agent is complete in Canada.

Accrual to this clinical study began in August 2001 after the protocol was approved by the Human Subjects Research Review Board of the U.S. Army Medical Research and Materiel Command, and our local institutional Research Ethics Board. The projected accrual was 65 patients annually, to achieve a total study population of 195 patients (156 eligible patients after allowing for attrition). The study has now been open for two years.

As outlined in the year 1 Annual Report, 40 patients were accrued in the first year. This was less than predicted because of unanticipated staff absences, equipment malfunction and replacement of aging radiotherapy treatment machines at this institution. Several measures were implemented to enhance accrual, and these have been successful. A total of 65 patients were entered onto the study in year 2, which is aligned with our target accrual. This represents a 62% increase over year 1 despite a 4 month interval from April 2003 to July 2003 when clinical and research activity at this institution was markedly curtailed because of Severe Acute Respiratory Syndrome (SARS). This high rate of accrual is expected to continue in year 3. However, it is expected that accrual will need to be extended for an additional 6-12 months beyond the original 3-year period to meet the overall target of 195 patients. This will not require any adjustment to the originally proposed budget, but an extension of the period for utilization of the funds.

Patients underwent prostate cancer oxygen measurements and prostate biopsies as described in the protocol. Two transrectal ultrasound-guided oxygen measurements were performed, followed by needle biopsies from the sites of the oxygen measurements to confirm

tumor. In addition, a third biopsy was obtained from the same region of the prostate for p53 gene sequencing and related immunohistochemical studies. Patients have tolerated these studies without difficulty apart from mild local discomfort. There have been no complications.

Prior to beginning accrual to this study in August 2001, a pilot study was performed to determine the feasibility of using this oxygen measurement technique in patients with prostate cancer. The pilot study was funded independently and was not part of the current award (DAMD17-01-1-0111). The pilot study has been accepted for publication, and the manuscript is contained in Appendix 1. The results validate the measurement technique and demonstrate that it provides a reliable indication of prostate cancer oxygenation. However, the analysis also showed that the biopsies from along the measurement tracks provide little clinically-relevant information, and that the reproducibility of the oxygen readings might be improved by making more oxygen measurements in each patient.

The study protocol was revised taking account of the results of the pilot study. The revised protocol specifies 4 oxygen measurement tracks. The biopsies from along the measurement tracks have been eliminated. A single biopsy will still be obtained from the region of the measurements for p53 DNA sequencing and immunohistochemical studies. It is anticipated that these changes will improve the quality of the data without impacting on the experience of patients participating in the study. The total number of needle punctures will remain unchanged at 5. The duration of the procedure will remain unchanged. There should be no difference in the very low risk of bleeding or infection (neither of which has been observed in the patients studied to date).

The University Health Network (UHN) Research Ethics Board (REB) approved this revision to the protocol on May 15, 2003. A letter summarizing the changes to the protocol, the revised patient consent form and the Letter of Approval from the UHN REB are contained in Appendices 2-4 respectively. The revision was submitted to Dr. Nrusingha Mishra on June 4, 2003 for consideration by the U.S. Army Human Subjects Review Board. To date, no response has been received. It is hoped that this review will be expedited so that patients participating in this study can be studied in an optimal manner.

As outlined in Section 4.1 of the protocol, the study includes patients participating in an independent randomized study of the anti-androgen bicalutamide 150 mg daily administered for a total duration of 5 months (3 months prior to, and 2 months concurrent with radiotherapy). Oxygen measurements will are made prior to the hormonal treatment. This randomized bicalutamide study is temporally closed to accrual pending review of other international bicalutamide studies where long-term (2 years or greater) use of this agent at this dose has been associated with a slightly higher than expected rate of death. Short-term use of bicalutamide as in our study has not been associated with an increased risk of adverse events. Therefore, it is anticipated that our bicalutamide study will re-open shortly and that our goal of determining the interaction between prostate cancer oxygenation and androgen ablation on patient outcome will not be effected.

A minor component of the project involves the use of the hypoxia marker EF5. As outlined in Section 4.6 of the protocol, this was to be administered to 30 patients in years 2 and 3 to evaluate the microscopic distribution of oxygen in prostate cancer, and differences in gene expression between oxic and hypoxic regions. This component of the proposed work has been revised to reflect significant development in the area of intrinsic hypoxia markers. These are normal proteins that are known to be up-regulated in the setting of hypoxia, and can be used in the same manner as EF5 to evaluate micro-regional aspects of tumor oxygenation. The intrinsic markers have several advantages, in that administration of an external agent prior to biopsy is not required and the immunohistochemical analysis can be done on previously-obtained paraffin embedded tissue. We will use the intrinsic markers carbonic anhydrase IX (CA-IX), glucose transporter (GLUT-1) and hypoxia inducible factor-1α (HIF-1α) to accomplish the goals of this

project (1-5). The analysis technique for the instrinsic markers will not differ significantly from that originally proposed for EF5, and we therefore do not anticipate any change to the budget.

The component of the project investigating the relationship between prostate cancer oxygenation, p53 status and downstream activation of p53 related pathways (section 4.5 of the protocol) are progressing as planned. The laser-capture micro-dissection, DNA sequencing and immunohistochemistry techniques have been developed, tested and refined. The patient samples will be processed as a batch once accrual to the study is complete to assure a consistent analysis approach.

Task 2. Follow-up (years 4-7)

Patients will be followed for a duration of 3.5 years after completion of accrual in order to realize the required 46 PSA relapses. Patients will be assessed clinically and have PSA measured at regular intervals as part of their routine medical care. The database will be updated on an ongoing basis to reflect current disease and patient status.

Patients who were accrued to the study in years 1 and 2 are being followed after the oxygen measurements and radiotherapy as outlined in section 4.8 of the protocol. Regular review of the patient data is performed to assure completeness and accuracy.

Task 3. Analysis (years 4 and 7)

The comparison of tumor oxygenation to other clinical and surgico-pathologic prognostic factors will be done after completion of accrual (early in year 4). The analysis of the influence of tumor hypoxia on outcome will be done after patients have been followed for an additional 3.5 years (mid year 7).

No analysis of the data has been undertaken.

Key Research Accomplishments

1. Ongoing patient accrual in alignment with that predicted in the original study proposal. Because of slower than expected accrual in year 1 and disruption in clinical and research activity in year 2 by Severe Acute Respiratory Syndrome (SARS), it is anticipated that accrual will need to be extended into year 4 to meet the target of 195 patients.
2. Revision of the oxygen measurement technique based on the results of our pilot study, to assure that the highest quality data are obtained. We are currently awaiting approval of the revised technique by the U.S. Army Human Subjects Review Board, and anticipate that this will be forthcoming shortly.
3. Revision of the technique for studying the microscopic distribution of hypoxia in prostate cancer based on evolving knowledge of intrinsic tissue markers of hypoxia.

Reportable Outcomes

None to date

Conclusions

This study of oxygenation in human prostate cancer continues to accrue patients. The accrual rate increased in year 2 and is now close to the value anticipated when the study was designed. Nevertheless, because of slower than expected accrual in year 1 and disruption in clinical and research activity in year 2 by Severe Acute Respiratory Syndrome (SARS), it is anticipated that accrual will need to be extended into year 4 to meet the target of 195 patients. The oxygen measurement technique has been revised based on our pilot study to assure that the highest quality data are being collected. We are currently awaiting approval of the revised technique by the U.S. Army Human Subjects Review Board, and anticipate that this will be forthcoming shortly. The microregional distribution of oxygen in prostate cancer biopsies will be studied using intrinsic markers of oxygenation rather than EF5 as described in the initial proposal. The molecular studies of p53 are proceeding as outlined in the proposal.

Overall, the aims of this study are progressing as planned and, apart from a 6-12 month in completing accrual, it is anticipated that all aspects of the work will be completed as initially proposed.

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Appendix 1

A Polarographic Electrode Study of Tumor Oxygenation in Clinically Localized Prostate Cancer

(Int J Radiat Oncol Biol Phys, In press, 2003)

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Abstract

Purpose: Hypoxia has prognostic and therapeutic significance in a number of human tumors. This report describes the oxygenation of clinically localized prostate cancer.

Materials and Methods: Intra-prostatic oxygen tension was measured using the *Eppendorf* electrode in 55 unanesthetized men with localized prostate cancer prior to radiotherapy. Measurements were made along two tracks through regions of suspected tumor in the prostate, and core needle biopsies were then obtained from the same regions.

Results: The median pO_2 ranged from 0.2 to 57.3 mm Hg, and the grand median pO_2 was 4.5 mm Hg. The percentage of oxygen readings <5 mm Hg (HP_5) ranged from 0% to 100%, with a median of 60%. The track 1 oxygen readings were higher than those from track 2. There was significant heterogeneity in the individual oxygen readings: the between- and within-tumor components accounted for 32% and 68% of the total variability respectively. However, the between-tumor variability in HP_5 significantly exceeded the within-tumor variability (61% vs. 39%). There was no association between oxygen values and clinical factors, including age, T-category, Gleason score, PSA, hemoglobin concentration or prior hormonal treatment. There was no difference in oxygenation between regions of tumor and normal prostate tissue determined from the core biopsies.

Conclusions: Localized prostate cancer is characterized by marked hypoxia and significant heterogeneity in oxygenation, similar to other human tumors. Normal prostate may contain regions of low oxygen concentration. HP_5 as determined in this study should adequately discriminate among patients with prostate cancer and allow the independent prognostic significance of oxygenation to be evaluated once the study matures.

Introduction

Tumor hypoxia is a common feature of a range of human malignancies, and has both prognostic and therapeutic significance. The degree of tumor hypoxia, as measured using polarographic electrodes, is an independent prognostic factor for survival in both cervical carcinoma [1-3] and head and neck cancer [4]. Randomized trials of strategies designed to improve tumor oxygenation or specifically target hypoxic cells have demonstrated survival benefits in patients with non small cell lung cancer [5] and head and neck cancer [6, 7]. The importance of hypoxia in prostate cancer is not known. However, low oxygen levels similar to those observed in other tumor types have been described in animal models of prostate cancer [8, 9], and in the only other polarographic electrode study to date of human prostate oxygenation [10, 11].

We have devised a polarographic electrode technique for measuring intra-prostatic oxygen levels in awake, unanesthetized men with localized prostate cancer. Core needle biopsies are obtained at the time of the measurements from the same locations in the prostate to verify the presence or absence of tumor. The ultimate objective of the study is to evaluate the independent prognostic significance of tumor oxygenation in patients with localized prostate cancer undergoing conformal radiotherapy. The aims of this report are to: 1) describe the measurement technique; 2) demonstrate that the technique is feasible and safe in a routine clinical setting and tolerated well by patients; 3) describe the oxygenation of clinically localized prostate cancer; 4) evaluate the heterogeneity of oxygen readings in prostate cancer; and 5) evaluate oxygenation in relation to clinical characteristics.

Materials and Methods

Patient Selection

Intra-prostatic measurements of oxygen tension were made between June 1999 and July 2000, using the *Eppendorf* polarographic electrode system, in men with clinically localized prostate cancer prior to high-dose conformal radiotherapy. Eligibility criteria were a histologic diagnosis of prostatic adenocarcinoma, clinical T1c, T2a/b or T3a disease (1997 TNM classification), no evidence of lymph node or distant metastases on CT scan of the abdomen/pelvis and bone scan, ECOG performance status 0-2, age < 80 years, a prior decision to treat with high-dose conformal radiotherapy and the ability to give informed consent.

Oxygen Measurements

Prior to receiving conformal radiotherapy for prostate cancer at our institution, patients routinely have three inert gold seeds inserted into the prostate under trans-rectal ultrasound (TRUS) guidance. These seeds serve as fiducial markers for radiation treatment planning and verification purposes. Oxygen measurements were made immediately prior to seed insertion as part of the same procedure in awake, unanaesthetised patients lying in the left lateral position. Prophylactic antibiotics were administered to minimize the risk of infection. All of the measurements were made by a single operator experienced in the use of TRUS-guided biopsy of the prostate (AT) to assure consistency of technique. Tumor location within the prostate gland was identified by palpation and TRUS, with the benefit of previous biopsy information. Under

direct TRUS guidance, the tip of a custom-manufactured fine-needle *Eppendorf* electrode, 300 μm in diameter and 17 cm in length, was introduced trans-rectally and positioned in the posterior prostate just beneath the capsule until the oxygen reading stabilized (usually less than one minute). Care was taken to minimize displacement or compression of the prostate gland with insertion of the ultrasound probe. Between 20 and 25 oxygen measurements were made 0.7 mm apart along a linear path through the tumor (track 1). The electrode was then retracted and a second series of 20 to 25 measurements was obtained immediately along a separate linear track through the tumor (track 2). The second measurement track was usually in the same region of the prostate as the first, and separated from the first by <1 cm. Movement of the needle through the tumor and the oxygen measurements along each track were controlled automatically by the *Eppendorf* machine, visualized on ultrasound, and recorded on videotape. Oxygen values <-3 mm Hg were assumed to be erroneous based on previous calibration experiments, and were deleted from the data set [12].

TRUS-guided core needle biopsies were obtained along each track after all of the oxygen measurements were completed, and immediately before insertion of the marker seeds. The biopsies, which measured 1 mm in diameter and 10-20 mm in length, were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned longitudinally at 3-5 μm intervals. The sections were stained with haematoxylin and eosin, and examined using light microscopy by an experienced genitourinary pathologist (JS) for the presence or absence of carcinoma. In addition, discontinuous regions of tumor and normal prostate tissue were mapped along the length of the biopsy from superficial to deep for comparison with the corresponding oxygen measurements.

The study was approved by the Clinical Trials Committee of the Princess Margaret Hospital, and the Human Subjects Review Committee of the Office of Research Services at the

University of Toronto. Informed written consent was obtained from all patients. The measurements added approximately 5 minutes to the duration of the routine marker seed insertion procedure. Patients were uncomfortable during the test because of the intra-rectal ultrasound probe, and experienced mild local pain with the measurements and biopsies. There were no complications from the procedure, including no bleeding and no infection.

Statistics

Tumor oxygenation was described by the median pO_2 and by the hypoxic proportion (HP_5), defined as the percentage of the 40 to 50 individual pO_2 measurements in each patient (pooled results from tracks 1 and 2) that were <5 mm Hg. The median pO_2 and HP_5 was also calculated separately for each track, and the results compared using the Wilcoxon matched-pairs signed rank-sum test [13].

The intrinsic variability of individual oxygenation measurements was estimated for comparison with other tumors [14-16]. The total pO_2 variance for the cohort (considering all measurements in all patients) was divided into a between-patient component and a within-patient component. The within-patient variance was divided again into a between-track component and a within-track component. The data were analyzed using a nested linear mixed model [17]. The track variable was nested within the patient variable. The between and within-patient variances were expressed as percentages of the total (within-patient + between-patient) variance.

The hypoxic proportion (HP_5) was used to evaluate oxygen status in relation to clinical prognostic factors. HP_5 has been shown to predict outcome in patients with cervix cancer [2] and was approximately normally distributed in this population of patients with prostate cancer. A

total of 40 individual oxygen measurements was predicted to yield a worst-case standard error in estimating HP_5 of 7.9% [18]. Doubling the number of individual measurements was predicted to reduce the standard error only minimally to 5.6%. Therefore, measurements were made along only two tracks to provide acceptable accuracy and reproducibility while minimizing the duration of patient discomfort and the general tolerability and acceptance of the procedure. To verify the adequacy of this approach, the between-patient and within-patient variance components were estimated for HP_5 assuming that each measurement track provided an independent estimate of HP_5 for a particular tumor.

The two-sample t-test was used to compare oxygenation when two independent groups were defined (age dichotomized about the median value of 70 years, T1c vs. T2/T3a, Gleason 6 vs. Gleason 7-8, prostatic volume dichotomized about the median value of 48 cm^3, hemoglobin concentration dichotomized about the median value of 146 g/l, and NAD vs. no NAD, positive biopsy vs. negative biopsy). One-way analysis of variance was used to compare oxygenation in the three clinical groups defined by PSA $\leq$4.0 vs. 4.1-10 vs. >10 ng/ml.

All reported p-values are 2-tailed. All statistical analyses were performed using SAS/STAT software, version 8 (Cary, North Carolina).

Results

Patient Characteristics

Prostate oxygenation was measured in 55 men with biopsy-proven prostate cancer. The measurements were made prior to any treatment in 46 cases, and the remaining 9 had received

neoadjuvant androgen deprivation (NAD) for a median duration of three months (range three weeks to eight months). The characteristics of the patients are summarized in Table 1.

Prostate Oxygenation

A total of between 40 and 50 individual oxygen readings was obtained along two tracks in each of the 55 patients, yielding a total of 2310 readings over 109 tracks (one patient had three tracks, and two patients had only one track for technical reasons). The median pO_2 in the 55 patients, taking all oxygen readings from both tracks into account, ranged from 0.2 to 57.3 mm Hg, and the grand median pO_2 (the median of the 55 median values) was 4.5 mm Hg. The HP_5 ranged from 0% to 100%, with a median of 60%. The distribution of these summary measures of oxygenation are illustrated in Figures 1(a) and 1(b) respectively. There was a systematic difference in the track 1 and track 2 oxygen readings, with track 1 being better oxygenated than track 2. The mean and median paired differences in median pO_2 (track 2 median pO_2 – track 1 median pO_2 in individual patients) were –0.8 and –1.7 mm Hg respectively (p=0.003), and the mean and median paired differences in HP_5 were 14.4% and 4.8% respectively (p=0.008). The oxygen measurements are summarized in Table 2.

Heterogeneity of Oxygen Readings

Evaluation of the 2310 individual oxygen readings from both tracks in all 55 patients yielded a between-patient variance of 32% and a within-patient variance of 68%. The within-patient variance was further divided into a between-track component of 24% and a within-track

component of 44%. The between-track component is attributable to the systematic difference in oxygenation between track 1 and track 2 that was described previously. There was no systematic difference in oxygen readings along the length of the tracks from superficial (sub-capsular) to deep.

To validate the use of only two measurement tracks to evaluate prostate cancer oxygenation, the variance component analysis was repeated for HP_5 assuming that each measurement track in each tumor provided an independent estimate of HP_5 for that tumor. There were 106 HP_5 values distributed among 53 tumors. The between-patient variance in HP_5 comprised 62% of the total, and the within-patient variance was 38%.

Oxygenation vs. Clinical Characteristics

The pooled HP_5, taking account of all oxygen readings along both measurement tracks, was used to compare oxygenation to clinical characteristics, and the results are shown in Table 3. There were trends towards greater hypoxia with advanced age, lower Gleason score, larger prostatic volume and lack of prior hormonal therapy. However, none of these associations reached statistical significance.

Given the systematic difference in oxygen readings between tracks 1 and 2, the relationship between HP_5 and the clinical characteristics of the patients was also analyzed separately for each track. For track 1, the only factor that was associated with significantly lower oxygen readings was the lack of prior NAD. The track 1 median HP_5 was 19% among the 9 patients who had received NAD, and 57.1% among the remaining patients who had oxygen measurements performed prior to any treatment for prostate cancer ($p=0.03$). For track 2, there

was no detectable relationship between HP_5 and any of the clinical variables, including the use of NAD.

Oxygenation vs. Pathology

A core needle biopsy was obtained along each of the *Eppendorf* electrode tracks immediately after the oxygenation measurements. The biopsies were 1 mm in diameter and ranged in length from 9.8 to 22.9 mm (median 14.2 mm). Although the measurement and biopsy tracks were aligned as closely as possible using ultrasound guidance, it was not possible to identify the *Eppendorf* track in the biopsy specimens. Both of the biopsies contained tumor in 34 cases, both were negative in 14 cases, and only one of the two biopsies contained tumor in the remaining seven cases. The overall median HP_5 (both tracks combined) was 69% for the 41 cases with at least one positive biopsy, and 50% when both biopsies were negative (p=0.5). The track 1 median HP_5 was 61.9% for the biopsy-positive cases, and 35.7% for those that were biopsy-negative (p=0.09). The corresponding track 2 median HP_5 values were 71.4% and 69.0% respectively (p = 0.87).

Discontinuous regions of tumor and normal prostate tissue were mapped along the length of the biopsy from superficial to deep for comparison with the corresponding oxygen measurements. The median lengths of tumor and normal prostate in each biopsy were 7.3 mm and 6.6 mm respectively. There was no difference in oxygenation between tumor and normal prostatic tissue (median HP_5 of 62% and 59% respectively). It was recognized that slight differences in the trajectory of the measurement and biopsy needles, despite identical entry points, might lead to greater spatial error at depth in the prostate. Furthermore, prostate cancer is

usually located in the sub-capsular peripheral zone of the gland. For these reasons, the analysis was repeated using only the most peripheral 10 mm of tissue. Again, there was no difference between the oxygen readings in tumor and normal prostate (median HP_5 of 65% and 58% respectively).

Discussion

The results of this study confirm that hypoxia of potential biologic significance exists in clinically localized prostate cancer. The grand median pO_2 of 4.5 mm Hg and the median HP_5 of 60% are similar to previously reported results in cervical carcinoma and head and neck cancer, tumor types in which oxygen level is an independent predictor of survival [1-4]. Most of our prostate tumors contained hypoxic regions. The median pO_2 values had a non-gaussian distribution as shown in Figure 1(a), and most were clustered in the narrow range <10 mm Hg. This implies that even small differences in median pO_2 may be indicative of potentially important differences in underlying tumor oxygenation. In contrast, HP_5 was approximately normally distributed between 0 and 100% and allowed greater discrimination among tumors. We found no apparent relationship between oxygenation and pre-treatment clinical prognostic factors (T-category, Gleason score or PSA). However there was a trend towards higher oxygen readings in patients who had received prior androgen ablation. This requires confirmation in a larger cohort of patients with longer follow-up.

There have been few other studies of human prostate cancer oxygenation, probably in part because of the relative inaccessibility of the gland to needle electrodes. Rasey *et al.* [19] reported hypoxic proportions of between 0 and 94% in four human prostate cancers based on

positron emission tomographic (PET) imaging of a fluorinated nitroimidazole compound that is taken up in regions of hypoxia. The only other oxygen electrode study in human prostate cancer was reported by Movsas *et al.* [10], and included 41 patients with clinically localized disease who were studied under spinal anaesthetic prior to prostate brachytherapy. Approximately 100 oxygen measurements along five needle tracks were obtained in each patient from the involved portion of the prostate. The overall median pO_2 was 6.3 mm Hg, which is similar to our result. Oxygenation was significantly better in patients younger than 62 years, and in those with T1 vs. T2-3 disease. A preliminary outcome analysis suggested that the ratio of prostate pO_2 to normal muscle pO_2 predicted biochemical failure following treatment [20].

The measurement of prostate cancer oxygenation prior to external beam radiotherapy is technically difficult because of the anatomic position of the prostate gland, and necessitates a compromise between the two fundamental goals of obtaining reliable and reproducible information about tumor oxygenation while at the same time minimizing patient discomfort and inconvenience. Our approach addresses these issues by coupling the oxygen measurements to the routine procedure of fiducial marker insertion that all of our patients with prostate cancer undergo prior to conformal radiotherapy. This allows trans-rectal access to the prostate gland for oxygen measurements without the need for an additional hospital visit or additional patient preparation (laxatives, prophylactic antibiotics). Current trans-rectal ultrasound and biopsy techniques allow the measurements and extra biopsies to be obtained in awake, unanesthetized patients with minimal discomfort and a low risk of side effects. The combination of previous diagnostic biopsy information, palpation of the prostate gland, ultrasound echogenicity and doppler blood flow was used to target regions of tumor, and was accurate in 75% of cases based on biopsies obtained from the same regions immediately after the measurements.

The *Eppendorf* electrode measures the average oxygen concentration in a volume of tissue at the tip of the electrode that might contain tumor cells, tumor interstitium, blood vessels and normal prostate gland. In addition, there is both spatial and time-dependent variation in oxygen concentration. These factors contribute to variability in oxygenation from region to region. The within-tumor variability of individual oxygen reading in prostate cancer accounted for 68% of the total sample variability. For comparison, Nordsmark *et al.* [15] described a within-tumor variance component of 75% in head and neck cancer, and 69% in soft tissue sarcoma. Wong *et al.* [16] reported the within-tumor component in cervix cancer to be 67% of the total variance. These results imply remarkable similarity in the intrinsic heterogeneity of individual oxygen readings in tumors of different types, despite variation in histology and patterns of growth. They also underscore the importance of obtaining multiple measurements in each tumor to reliably and reproducibly evaluate overall oxygenation. Repeat measurements done in the same manner at later times should yield the same result within the accuracy limits of the test, assuming no change in the true underlying oxygen status of the tumor. It can be estimated that 40 individual oxygen readings in each tumor will produce a standard error in the estimation of HP_5 of 7.9%, and that increasing the number of measurements reduces the standard error in a diminishing fashion according to an inverse square-root function [18]. Therefore, 40 measurement were chosen in this study as a compromise between reproducibility and patient acceptance of the procedure. To assess the validity of this approach, the variance-component analysis was repeated for HP_5, assuming that each track provided an independent estimate of HP_5 for a particular tumor. The between-tumor component accounted for 61% of the total sample variance in HP_5, and significantly exceeded the within-tumor component. Therefore, HP_5 as measured in this

study should adequately discriminate among patients for the propose of evaluating the independent prognostic significance of oxygenation in prostate cancer.

We found the oxygen readings to be influenced by the sequence in which the measurements were made. The track 1 pO_2 readings were significantly higher than the track 2 readings, although there were no systematic differences in oxygenation along the length of the measurement tracks. This is in contrast to our experience in cervix cancer, where no systematic differences were observed among measurements from different tracks in the same tumor [16]. It raises concern that the process of making measurements along the first track might have influenced the results obtained from the second track. Possible explanations include pressure from the intra-rectal ultrasound probe, vascular disruption, reactive vasoconstriction, bleeding or edema. While this observation implies the need for caution when pooling oxygen measurements from multiple tracks in patients with prostate cancer, the magnitude of the difference between tracks was small (mean paired difference in HP_5 of 14.4%, median paired difference of 4.8%) and therefore probably of minimal clinical significance. Movsas *et al.* [10, 11] did not specifically comment on differences in the oxygen readings among measurement tracks in their patients with prostate cancer.

There was a trend towards lower oxygen readings if the accompanying core needle biopsies from the region of the measurements contained tumor. However, the relationship failed to achieve statistical significance. Early prostate cancer is characterized by infiltrative, multifocal growth, and discontinuous regions of tumor and normal prostate were frequently seen in the biopsies. To more accurately evaluate the oxygenation of prostate cancer as distinct from normal prostatic tissue, the location of tumor and normal prostate was mapped along the length of each biopsy and correlated with the corresponding oxygen readings. No difference in oxygenation was

detected between tumor and normal tissue (median HP_5 of 62% and 59% respectively). The measurement tracks and biopsies were closely aligned using ultrasound guidance but it was not possible to reliably identify the electrode track in the biopsy specimens. It was recognized that slight differences in the trajectory of the measurement and biopsy needles, despite identical entry points, might lead to greater spatial error at depth in the prostate. Therefore, the mapping analysis was repeated using only the most peripheral 10 mm of the prostate biopsies. This is likely to encompass most of the tumor, which is usually located in the sub-capsular peripheral zone of the gland. Despite this more rigorous analysis, there was no difference in oxygenation between the regions. These results suggest that the normal prostate gland may contain regions that are poorly oxygenated, and comparable in this respect to tumor. This will need to be addressed in future studies. An important question is whether or not "contamination" of the tumor oxygen readings by normal tissue measurements, though similar in value, might influence the prognostic value of the procedure. Movsas *et al.* [20], in a preliminary analysis, described an association between oxygen measurements made randomly in the involved region of the prostate (without pathologic confirmation of tumor at the measurement site) and outcome following radiotherapy, suggesting that more precise localization of the electrode in tumor may not be necessary.

There is mounting evidence that hypoxia exists in prostate cancer, as it does in other human tumors. Hypoxia promotes a range of genetic alterations implicated in malignant progression [21], including the selection of cells with mutations of the p53 tumor suppressor gene [22]. In addition, hypoxia is an important stimulus of angiogenesis, and several studies have shown increased expression of vascular endothelial growth factor (VEGF) and other angiogenic proteins in prostate tumors [23-25]. A correlation between needle electrode oxygen measurements and VEGF

expression has been demonstrated in patients with prostate cancer prior to prostatectomy [26]. Both p53 mutations and high levels of angiogenesis have been linked to early tumor progression following radiotherapy of prostate cancer, and lower overall patient survival [27-29]. Hormonal therapy of androgen-sensitive prostate cancer may inhibit angiogenesis [30, 31] and lead to improved tumor oxygenation [32]. These observations, together with preliminary needle electrode results [20], support the hypothesis that hypoxia is an important cause of radiation treatment failure in prostate cancer. Our study continues to accrue and mature, and will eventually allow us to assess the interaction between hormonal treatment and oxygenation as well as the independent prognostic significance of oxygen measurements in this disease.

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Table 1. Patient characteristics

Number	55
Median age (range)	70 yrs (52-79)
Clinical T-category*	
T1c	15
T2a	34
T2b	4
T3a	2
Gleason score	
6	15
7/8	40
Initial PSA	
≤4 ng/ml	9
4.1-10	25
>10	21
Prior NAD	
Yes	9
No	46
Median prostate volume† (range)	48 cm^3 (14-179)
Median Hgb (range)	146 g/l (115-187)

* 1997 TNM Classification

† From trans-rectal ultrasound

Hgb Hemoglobin concentration

NAD Neoadjuvant androgen deprivation

Table 2. Summary of oxygen readings

	Median pO_2		HP_5	
	Range	**Grand Median**	**Range**	**Median HP_5**
Overall (Track 1 and 2)	0.2-57.3 mm Hg	4.5 mm Hg	0-100%	60%
Track 1	1.5-75.4	4.9*	0-100	55†
Track 2	0-70.4	3.0*	0-100	71†

* Mean paired difference –0.8 mm Hg, median paired difference –1.7 mm Hg, p=0.003

† Mean paired difference 14.4%, median paired difference 4.8%, p=0.008

HP_5 Hypoxic proportion (proportion of oxygen readings <5 mm Hg)

Table 3. Univariate analysis of HP_5 vs. clinical characteristics

	Cases	Overall median HP_5 (%)
Age		
≤70 years (median)	32	56
>70 years	23	69
T-category		
T1c	15	59
T2/T3a	40	61
Gleason score		
6	15	69
7-8	40	56
PSA		
0-4.0	9	50
4.1-10	25	64
>10	21	54
Hgb		
≤146 g/l (median)	18	54
>146 g/l	18	67
Unknown	19	59
Prostate volume		
≤ 48 cm^3 (median)	32	55
> 48 cm^3	23	69
Prostate biopsy		
Positive	41	69
Negative	14	50
Prior NAD		
Yes	9	48
No	46	67

Hgb Hemoglobin concentration

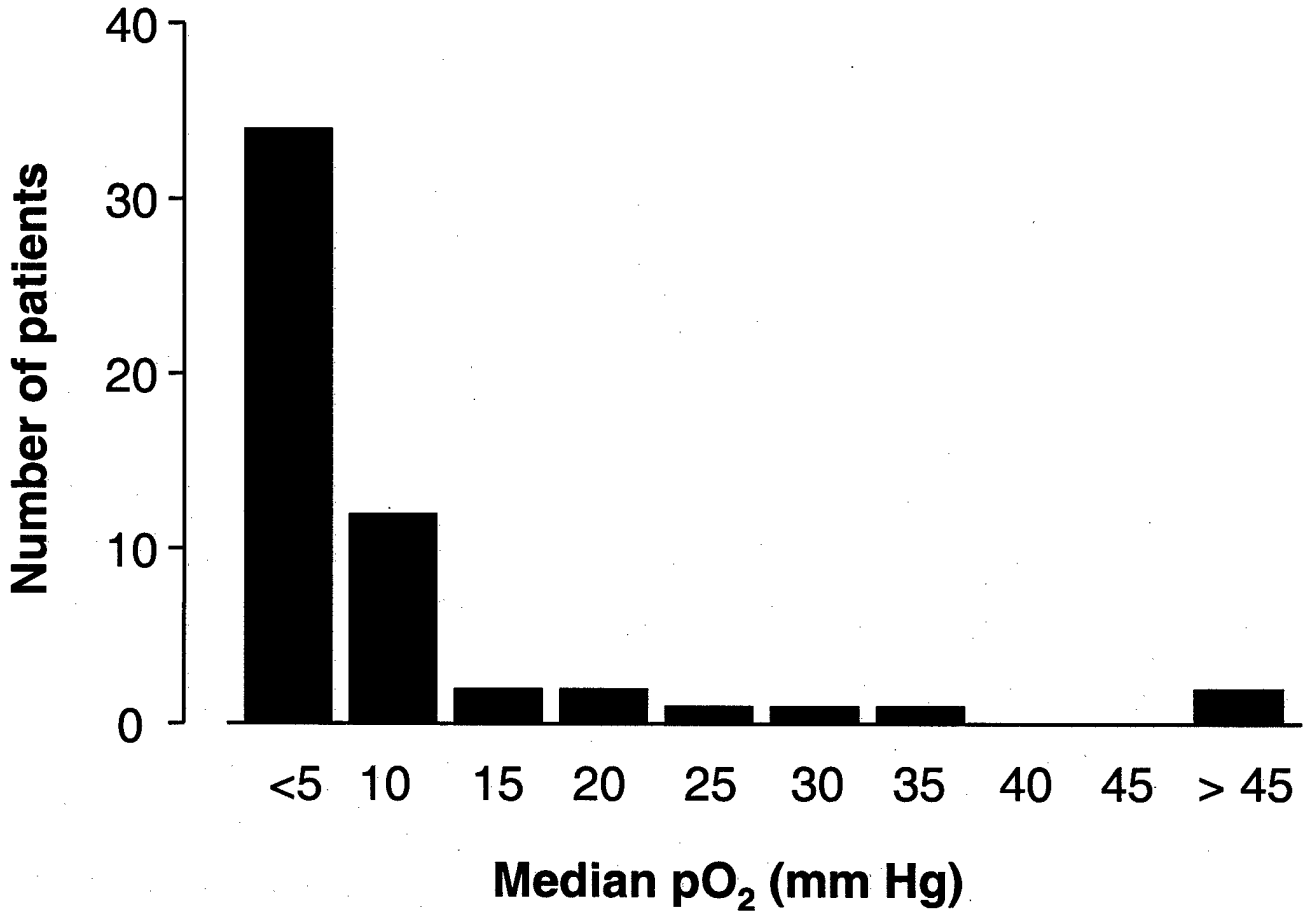
HP_5 Hypoxic proportion (proportion of oxygen readings <5 mm Hg)

NAD Neoadjuvant androgen deprivation

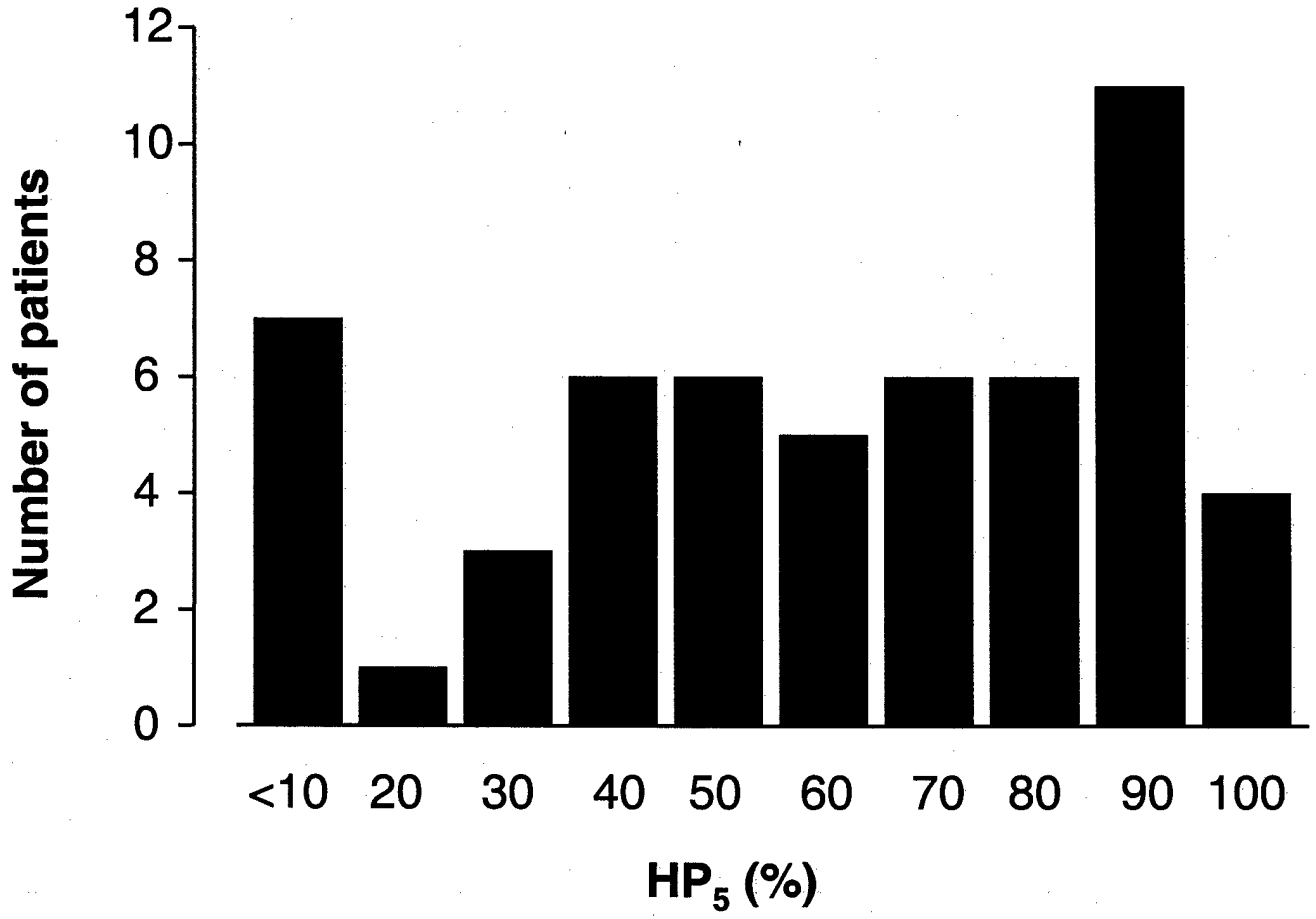
Figure Legend

Figure 1a. Distribution of median pO_2 in 55 patients with localized prostate cancer

Figure 1b. Percentage of oxygen measurements <5 mm Hg (HP_5) in 55 patients with localized prostate cancer



Parker - Figure 1a



Parker - Figure 1b

Appendix 2

April 19, 2003

Dr Ron Heslegrave
Chair, Oncology Research Ethics Board
University Health Network

Dear Dr. Heslegrave

Re: Trans-Rectal Tumor Oxygen Measurements in Patients with Clinically Localized Prostate Cancer – REB # 00-0443-C

Please find attached an amendment to the above protocol. This study, which is supported by the U.S. Army, has been active at Princess Margaret Hospital since August 2001. It involves measuring prostate cancer oxygenation along two linear tracks through the prostate gland under ultrasound guidance using a special needle electrode system. Approximately 20 individual oxygen measurements are made along each track, yielding a total of about 40 measurements per patient. Standard transrectal ultrasound guided biopsies are then made along each of the needle tracks to assure that the measurements are made within tumor. A third biopsy is obtained from the same region for tumor banking and to evaluate hypoxia- related differences in gene expression. To date, approximately 150 patients have been accrued to this study and an earlier pilot study that employed the same technique. The measurements have been well tolerated and there have been no complication.

The data from the earlier pilot study have now been analyzed. The biopsies from along the measurement tracks provide little clinically-relevant information. Furthermore, the results from the pilot study suggest that the reproducibility of the oxygen results might be improved by making more oxygen measurements in each patient.

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The study protocol has been revised taking account of the results of the pilot study. The revised protocol involves increasing the number of oxygen measurement tracks from 2 to 4. The biopsies from along the measurement tracks have been eliminated. A single biopsy will still be obtained from the region of the measurements for tumor banking. These changes will improve the quality of the data without impacting on the experience of patients participating in the study. The total number of needle punctures remains unchanged at 5. If anything, it is expected that patient acceptance of the procedure will actually increase as a result of these changes because experience to date suggests that the needle electrode measurements are better tolerated than the biopsies. The overall duration of the procedure will not change. There should be no difference in the very low risk of bleeding or infection (neither of which has been observed in patients studied to date).

Another related study (UHN REB # 00-0430-C) looks at the effect of hormonal treatment on prostate cancer oxygenation. This involves a second series of oxygen measurements in consenting patients after 2 months of hormonal treatment with bicalutamide. The oxygen measurement technique will be changed for this study as well, so that patients experience the same procedure on both occasions.

I trust that these changes will meet with your approval and that they can be approved in an expeditious fashion. I would be pleased to discuss them with you at any time. The revision will also be submitted to the U.S. Army, who is sponsoring the study.

Yours sincerely,

Michael Milosevic, MD, FRCPC
Attachement

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Princess Margaret Hospital

University Health Network

Appendix 3

Title: A Study of Transrectal Tumor Oxygen Measurements in Patients with Clinically Localized Prostate Cancer

Principal Investigator: Dr. Michael Milosevic
Princess Margaret Hospital
416-946-2124

Patient Information & Consent Form
Sponsor: United States Army Medical and Research Command

INTRODUCTION

You are being invited to volunteer as a participant in a research study, also known as a clinical trial. Before agreeing to participate, please read this information carefully and ask any questions you wish. This study is designed to evaluate the value of using a special fine-needle electrode to measure the amount of oxygen in prostate cancer tumors.

You have been diagnosed with prostate cancer and have decided with your physician to be treated with high-dose conformal radiotherapy. To be treated with high-dose conformal radiotherapy, 3 small marker seeds are inserted into the prostate before beginning the treatment. This is a standard part of planning conformal radiation, and provides assurance during treatment that the radiation is being accurately delivered to the prostate each day. The seeds will be inserted using a special needle that is introduced through the rectum in much the same way as biopsies of the prostate are often done. A small ultrasound device will be placed in the rectum at the same time to guide the positioning of the seeds.

DESCRIPTION OF STUDY

This study will enroll 195 patients at this site, Princess Margaret Hospital.

The study is designed to evaluate the value of using a special fine-needle electrode to measure the amount of oxygen in prostate cancer tumors. Tumor oxygen content may be an important factor that influences the effectiveness of radiotherapy and other treatments. These

measurements will be done immediately prior to insertion of the marker seeds described above, at the same clinic visit. The measurement needle will be inserted into the prostate tumor through the rectum under ultrasound guidance. Measurements will be made at four locations in the tumor. The measurements will be followed by a single biopsy of your prostate tumor. The biopsy will be used to be sure that the measurements were made in tumor, and to study the genetic makeup of your tumor. Genetic changes may be more likely to occur in tumors that are low in oxygen and may influence the effectiveness of radiation and other treatments. In addition, 5 ml of blood will be obtained and used to determine whether or not the oxygen status of a tumor can be determined directly from a blood test. The biopsy and blood will be stored indefinitely in a central location at Princess Margaret Hospital until these studies are performed, which may be several months or years in the future. The biopsy and blood will be used for these research purposes only.

The marker seeds for radiation treatment will be inserted in the usual way after all oxygen measurements and the biopsy has been completed. The entire procedure will take less than 15 minutes. Participation in the study will not delay the start of your radiotherapy.

After your radiotherapy has been completed, you will be assessed at Princess Margaret Hospital by your doctor at regular intervals. No additional visits to the hospital will be required as part of this study. In order to determine whether or not the tumor in your prostate was eradicated with radiotherapy, you will be asked to have another biopsy of the prostate 2 years after completing radiotherapy. This will be done in the same fashion as the other biopsies, using an ultrasound probe and needle inserted into the rectum. However, oxygen measurements will not be made.

BLOOD AND TUMOR SAMPLES

During this study, you will be asked to provide a blood sample and a biopsy of your prostate cancer. These samples will be used to determine whether the oxygen status of prostate cancer can be determined from a blood test, and to study how oxygen influences the genetic makeup of prostate cancer. These samples may also be used for research purposes that are currently unknown. There is a chance that the samples that you are donating as part of this study may be used in other research studies and may have some commercial value. Should your donated samples lead to the development of a commercial product, Princess Margaret Hospital will own it and may take action to patent and license the product. Princess Margaret Hospital does not intend to provide you with any compensation for your participation in this study nor for any future value that the sample you have given may be found to have. You will not receive any notice of future uses of your samples. You may agree to participate in the oxygen study, but refuse to have your blood and tumor tissue used for research.

BENEFITS

The information derived from this study will not be of benefit to you. It may improve the ability of doctors to treat patients with prostate cancer more effectively in the future. You will not be

informed of the results of the oxygen measurements or genetic studies, but you will be told whether or not tumor is present in your prostate 2 years after completing radiotherapy based on the biopsies obtained at that time.

RISKS

All patients who undergo conformal radiotherapy for prostate cancer at Princess Margaret Hospital are required to have 3 small marker seeds inserted into the prostate through the rectum before beginning the treatment. This is a standard part of planning conformal radiation, and provides assurance during treatment that the radiation is being accurately delivered to the prostate each day. You will need to have this done regardless of whether or not you participate in this study. The marker seed insertion is associated with local discomfort, and there may be a small amount of blood in your urine or semen for a few days after the procedure. You will be asked as part of routine care to take antibiotics for 2 days around the time of the marker seed insertion. The risk of serious infection or bleeding is less than 5%.

The oxygen measurements and the biopsy that are being done in this study may produce a small amount of additional discomfort and may also slightly increase the risk of bleeding or infection. The blood specimen will be obtained from a vein in your arm, and this may cause local discomfort. In addition, bleeding, bruising or infection may develop at the site of the needle puncture.

VOLUNTARY PARTICPATION

Your participation in this study is voluntary. You may stop taking part in this study at any time and without giving any reason. If you do decide to stop participating, this will not have an influence on any further medical treatment that you need or result in a loss of benefits. Your doctor also has the right to end your participation in this study, if it is in your best medical interests or if you are unable to comply with study procedures for any reason.

Before you make a decision about further participation in this study, the study doctor is available to answer any questions you may have and to explain the study. Allow yourself as much time as you need to think through your decision. If you then decide that you still wish to take part, your doctor will ask you to confirm in writing that you have read and understand this patient information, that all your questions have been answered completely and that you wish to continue with the study. You will be informed of any and all significant new findings that might develop during the course of this study, which could affect your willingness to continue in the study.

COMPENSATION FOR INJURY

Should you be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you, for that injury. You will not receive any injury

compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with your physician or with Dr. Michael Milosevic, the Principal Investigator for this study.

CONTACT INFORMATION

If you have any questions about the study, please call Dr. Michael Milosevic, the Principal Investigator, at 416-946-2124 or Dr. Mary Gospodarowicz, Chair of the Department of Radiation Oncology, at 416-946-4421. If you have any questions about your rights as a research subject, please call Dr. Ronald Heslegrave, Chair of the University Health Network Research Ethics Board, at 416-340-4557. In the event of a research-related injury, please call Dr. Jolie Ringash, the Medical Monitor for this study, at 416-946-2126.

DATA PROTECTION / CONFIDENTIALITY

The confidentiality of your personal information and medical record will be maintained, and will not be affected by participation in this study. The doctors at Princess Margaret Hospital who are conducting the study will examine your hospital chart and other records. Representatives of the U.S. Army Medical Research and Material Command, the sponsor of this study, may also examine research records as part of their responsibility to protect human subjects involved in research. All information about your case that is relevant to the study will be stored in a confidential database that will be maintained indefinitely at Princess Margaret Hospital. The blood and tumor samples will be stored in a locked location that is accessible only to the study doctors. The samples will not leave Princess Margaret Hospital. The samples will be stored until they are used or are no longer needed by the doctors performing the study. Any remaining samples will then be destroyed. At no point will your name or any other identifying feature appear in any publication based on this study.

Information about your participation in the study will be forwarded to the United States Army Medical and Research Command as an additional guarantee of your safety as a study participant. The information that is sent to the Army will be stored in a confidential database, and may include your name, address and United States social security number (if available). The intent of the database is twofold: 1) to readily answer questions about your participation in research sponsored by the Army, and 2) to ensure that the Army can exercise its obligation to ensure that you are adequately warned of risks and to provide new information as it becomes available. The information will be used solely to protect your rights as a patient participating in this study. It will be stored for a minimum of 75 years. The access to this database is strictly regulated to assure the highest degree of confidentiality at all times.

CONSENT

I have discussed this study with my physician, Dr._____, and any questions that I have about it have been answered. I know that I can also discuss this study with the Principal Investigator, Dr. Michael Milosevic (416-946-2124) or with the Chief of the Department of Radiation Oncology, Dr. Mary Gospodarowicz (416-946-4421). I may discuss my rights as a patient with Dr. Ronald Heslegrave (416-340-4557), who has no involvement with this study. I agree to participate in this study, and to have the oxygen measurements performed:

Yes_____ No_____

As a participant in this study, I voluntarily donate any and all blood and tumor samples. These samples will be used to determine whether the oxygen status of prostate cancer can be determined from a blood test, and to study how oxygen influences the genetic makeup of prostate cancer. They may also be used by Princess Margaret Hospital for research purposes that are currently unknown. There is a possibility that the samples that I am donating under this study may be used in other research studies and may have some commercial value. Should my donated samples lead to the development of a commercial product, Princess Margaret Hospital will own it and it is possible that it will be patented and licensed by Princess Margaret Hospital. Princess Margaret Hospital does not intend to provide me any compensation for this and will not give me any notice of future uses of my samples.

Yes_____ No_____

Patient Signature: _____ Patient name: (printed)_____

Patient Address: _____

_____

_____

_____ _____

Person obtaining consent: (signature) Date

_____

PMH RMP Version 22 April 2003 PMH REB Version 15 May 2003 US ARMY Version TBD	Patient Initials: _____ Person obtaining consent Initials: _____

Person obtaining consent: (printed)

Physician Signature: _____ Physician name: (printed)_____

Date: _____

University Health Network
Toronto General Hospital Toronto Western Hospital Princess Margaret Hospital

University Health Network Research Ethics Board
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May 15, 2003

Dr. Mike Milosevic
Princess Margaret Hospital
Rm 5-917
Department of Radiation Oncology

Dear Dr. Milosevic:

RE: **UHN REB#: 00-0443-C**
A Study of Transrectal Tumor Oxygen measurements in Patients with Clinically Localized Prostate Cancer

This is to inform you that the amendment (dated April 19, 2003) and the revised consent form (dated April 22, 2003) have been approved for implementation through expedited review by the University Health Network Research Ethics Board.

Best wishes for the successful completion of your project.

The signature below confirms our attestation to all information noted in the footer of this document.

Sincerely,

Sarah Warden, M.Sc.
Research Ethics Coordinator

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